

Amendments to the Specification

Please cancel the title on page 1 of the specification and replace it with the following title: "CANINE B7-2 PROTEINS, COMPOSITIONS AND USES THEREOF"

On page 1, following the title of the invention, please replace the previously submitted "CROSS-REFERENCE TO RELATED APPLICATIONS" paragraph with the following paragraph:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Divisional of pending prior National Phase Filing U.S. Patent Application Serial No. 09/646,561, filed September 19, 2000, entitled "Canine and Feline B7-2 Nucleic Acid Molecules and Uses Thereof"; which claims priority to international PCT Application No. PCT/US99/06187, filed March 19, 1999, entitled "Novel Forms of T Cell Costimulatory Proteins, Nucleic Acid Molecules, and Uses Thereof"; which is a continuation-in-part of U.S. Application Serial No. 09/062,597, filed April 17, 1998, entitled "Novel Forms of T Cell Costimulatory Proteins, Nucleic Acid Molecules, and Uses Thereof"; which claims priority to U.S. Provisional Application Serial No. 60/078,765, filed March 19, 1998, entitled "Novel Forms of T Cell Costimulatory Proteins, Nucleic Acid Molecules, and Uses Thereof".--

On page 18 of the specification , please replace the paragraph spanning lines 1-16 with the following paragraph:

Furthermore, it is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid sequences. These computer programs include various known methods to determine the percentage identity and the number and length of gaps between hybrid nucleic acid molecules. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, the Wisconsin Package Version 9.0 sequence analysis software, available from Genetics Computer Group (GCG), Madison, WI; DNASIS®

(DNAsis)TM, available from Hitachi Software, San Bruno, CA; and MACVECTOR[®] (MacVector)^{[[TM]]}, available from the Eastman Kodak Company, New Haven, CT. A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the GAP program with pair-wise comparisons within the Wisconsin Package Version 9.0 sequence analysis software, available from Genetics Computer Group (GCG), Madison, WI, hereinafter referred to as default parameters.

Please replace the paragraph spanning page 25, lines 21-31, through page 26, lines 1-6, with the following paragraph:

Percent identities between amino acid or nucleic acid sequences can be determined using standard methods known to those of skill in the art. It is known in the art that methods to determine the percentage identity and the number of gaps are substantially similar when different methods for determining sequence similarity are used and when the degree of similarity is greater than 30% amino acid identity, as described by Johnson et al., *J. Mol. Biol.*, vol. 233, pages 716-738, 1993, and Feng et al., *J. Mol. Evol.*, vol. 21, pages 112-125, 1985. Preferred methods to determine percentage identities between amino acid sequences and between nucleic acid sequences include comparison using various computer programs such as the Wisconsin Package Version 9.0 sequence analysis software, available from Genetics Computer Group (GCG), Madison, WI; DNASIS[®] (DNAsis)TM program, available from Hitachi Software, San Bruno, CA; or the MACVECTOR[®] (MacVector)TM program, available from the Eastman Kodak Company, New Haven, CT. A preferred method to determine percentage identities between amino acid sequences and between nucleic acid sequences includes using the DNAsisTM computer program with the following settings: the gap penalty set at 5; the number of top diagonals set at 5; the fixed gap penalty set at 10; the k-tuple set at 2; the window size set at 5 and the floating gap penalty set at 10.

Please replace the paragraph spanning page 52, lines 24-31, through page 53, lines 1-2, with the following paragraph:

To identify full-length B7-1 clones, the second PCR product was used to generate an about 545 base pair DNA fragment. The fragment was then labeled with ^{32}P and used as a probe to screen the canine PBMC cDNA library. Hybridization was done at about 68°C in 6XSSC, 5X Denhardt's solution, 0.5% SDS, 100 $\mu\text{g/ml}$ of salmon sperm DNA and yeast tRNA. The blot was washed two times, for about 30 minutes per wash, at 55°C in 1XSSC, 0.1%SDS. Positive clones were isolated and the cDNA inserts were sequenced for both strands using vector flanking primers and gene-specific internal primers. Sequence analysis was performed with DNASIS[®] (DNAsis)[™] using the alignment settings of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; k-tuple set at 2; window size set at 5 and floating gap penalty set at 10.

On page 55 of the specification, please replace the paragraph spanning lines 20-25 with the following paragraph:

To identify full-length B7-2 clones, the 322-bp PCR product was labeled with ^{32}P and used as a probe to screen the canine PBMC cDNA library. Hybridization was performed as described in Example 1. Positive clones were isolated and the cDNA inserts were sequenced for both strands using vector flanking primers and gene-specific internal primers. Sequence analysis was performed with DNASIS[®] (DNAsis)[™] using the settings described in Example 1.

On page 59 of the specification, please replace the paragraph spanning lines 1-27 with the following paragraph:

C. Stable expression of recombinant canine B7-1 and B7-2 in mammalian cells carrying the recombinant plasmids, pCMV-nCaB7-2₁₈₉₇ or pCMV-nCaB7-1₁₃₈₅ was demonstrated by introducing these plasmids into Chinese Hamster Ovary cells (CHO, available from ATCC, as follows. Briefly, six-well polystyrene tissue culture plates were seeded with approximately 5×10^5 /well in 2 ml of MEM, available from Life Technologies, supplemented with 100 mM L-glutamine, gentamicin, and 10% FBS (TCM). Cells were grown to about 80% confluence (about 18 hrs). The recombinant molecules to be transfected were purified using the 5' Prime to 3' Prime Kit, available from 5' to 3', Inc., Boulder, CO, as per the manufacturer's instructions. The recombinant plasmids were linearized using the restriction enzyme *PvuI*. The

plasmid pcDNA3, available from Invitrogen, which contains the neomycin resistance gene, was linearized with the restriction enzyme *EcoRI*. Approximately 2 µg of pCMV-nCaB7-2₁₈₉₇ or pCMV-nCaB7-1₁₃₈₅, were mixed separately with about 2 ng of pcDNA3 in about 100 µl OPTIMEM® (OptiMEM) medium, available from Life Technologies. About 10 µl LIPOFECTAMINE™ (Lipofectamine), available from Life Technologies, was mixed with about 100 µl OptiMEM. The plasmid mixture was then added to the Lipofectamine mixture and incubated at room temperature for about 45 min. After incubation, about 800 µl of OptiMEM was added, and the entire mixture was overlaid onto the CHO cells that had been rinsed with OptiMEM. Cells were incubated for about 5 hours at 37° C, 5% CO₂, 95% relative humidity. Approximately 1 ml of TCM with 20% FBS was added, and the cells were incubated overnight. The media was changed after about 24 hr. About 72 hr post transfection, the cells were split 1:4 and put into selection TCM containing about 400 Fg/ml geneticin (G418), available from Life Technologies. The media was changed every 3-5 days. After several weeks, G418-resistant colonies were trypsinized using cloning cylinders, and the cells were plated into 24 well plates. The resulting recombinant cells are referred to herein as CHO-pCMV-nCaB7-2₁₈₉₇ and CHO-pCMV-nCaB7-1₁₃₈₅, respectively. The recombinant cells were then expanded for testing.

On page 61 of the specification, please replace the paragraph spanning lines 3-13 with the following paragraph:

A *Felis* mitogen activated PBMC cDNA library was constructed in the Uni-ZAP® XR vector using the methods described above in Example 1. As a first step in the isolation of full-length feline B7-2 cDNA, a 322-bp PCR fragment encoding a portion of feline B7-2 was amplified from the cDNA library using the two degenerate primers described above in Example 2. To identify full-length B7-2 clones, the 322-bp PCR product was labeled with ³²P and used as a probe to screen the feline PBMC cDNA library using the hybridization conditions described above in Example 1. Positive clones were isolated and the cDNA inserts were sequenced for both strands using vector flanking primers and gene-specific internal primers. Sequence analysis was performed with DNASIS® (DNAsis)[™] using the settings described above in Example 1.

Please replace the paragraph spanning page 66, lines 19-31, through page 67, lines 1-12, with the following paragraph:

C. Stable recombinant cells individually expressing recombinant plasmids pCMV-nFeB7-2₉₉₉ and pCMV-nFeB7-1₈₇₉ were established in mouse L-M (TK-) cell fibroblasts (L cells, available from ATCC) as follows. Briefly, six-well polystyrene tissue culture plates were seeded with approximately 5×10^5 cells /well in 2 ml of DMEM, available from Life Technologies, supplemented with 100 mM L-glutamine, gentamicin, and 10% FBS (L-TCM). Cells were grown to about 80% confluence (about 18 hr). The recombinant plasmids to be transfected were purified using the 5 Prime to 3 Prime Kit as per the manufacturer's instructions. The recombinant plasmids were linearized with the restriction enzyme *PvuI*. The plasmid PMLBTK, available from ATCC, which contains the thymidine kinase gene was also linearized with *PvuI*. Approximately 2 µg of recombinant plasmid DNA and 0.2 µg of PMLBTK were mixed with about 100 µl OPTIMEM® (OptiMEM) medium, available from Life Technologies. About 10 µl LIPOFECTAMINE™ (Lipofectamine), available from Life Technologies, was mixed with 100 µl OptiMEM. The plasmid mixture was then added to the Lipofectamine mixture and incubated at room temperature for about 45 min. After incubation, about 800 µl of OptiMEM was added, and the entire mixture was overlaid onto the L cells that had been rinsed with OptiMEM. Cells were incubated for 5 hours at 37° C, 5% CO₂, 95% relative humidity. Approximately 1 ml of L-TCM with 20% FBS was added, and the cells were incubated overnight. The media was changed after about 24 hr. About 72 hr post transfection, the cells were split 1:4 and put into selection L-TCM containing 1 X HAT, available from Sigma. The media was changed every 3-5 days. After several weeks, HAT-resistant colonies were trypsinized using cloning cylinders, and the cells were plated into 24 well plates. The resulting recombinant cells are referred to herein as L-pCMV-nFeB7-2₉₉₉ and L-pCMV-nFeB7-1₈₇₉ respectively. The recombinant cells were then expanded for testing.

On page 70 of the specification, please replace the paragraph spanning lines 5-12 with the following paragraph:

Sequence analysis was performed with DNASIS® (DNAsis)™ using the alignment settings of: gap penalty set at 5; number of top diagonals set at 5; fixed gap penalty set at 10; k-tuple set at 2; window size set at 5 and floating gap penalty set at 10. At the amino acid level, PCaCTLA4₂₂₃ shared 88.5%, 88.2%, 87.4%, 76.7%, and 76.2% of identity with the CTLA4

proteins of rabbit, bovine, human, mouse, and rat, respectively. PFeCTLA4₂₂₃ shared 88.8%, 87.9%, 86.9%, 77.6%, and 77.1% of identity with the CTLA4 proteins of rabbit, human, bovine, mouse, and rat, respectively. PCaCTLA4₂₂₃ and PFeCTLA4₂₂₃ shared the highest identity (97.2%).

After page 85 of the specification, please insert the following new page containing the Abstract: